Modulating Hippocampal Output in the Pilocarpine Model of Epilepsy by β-adrenoceptor Activation

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von

Sabine Grosser, M.Sc. Biophysik

Prsident der Humboldt-Universitt zu Berlin:

Prof. Dr. Jan-Hendrik Olbertz

Dekan der Lebenswissenschaftlichen Fakultt:

Prof. Dr. Richard Lucius

1. Gutachter Prof. Dr. Joachim Behr
2. Gutachter Prof. Dr. Susanne Schreiber
3. Gutachter Prof. Dr. Imre Vida

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"Nothing in life is to be feared, it is only to be understood."

Marie Curie

Contents

Nomenclature

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ACSF	artificial cerebrospinal fluid
β-AR	β-adrenergic receptor
CA	cornu ammonis
CaMKII	CA^{2+} /calmodulin-dependent protein kinase II
cAMP	3',5'-cyclic AMP
fEPSP	excitatory postsynaptic field potentials
EPSC	excitatory postsynaptic current
GABA	γ-aminobutric acid
I/O curve	input-output curve
i.p.	intraperitoneally
ISO	isoproterenol
lEC	lateral entorhinal cortex
LTP	long-term potentiation
MEA	multi-electrode array
mEC	medial entorhinal cortex
NE	norepinephrine
NMDA	N-methyl-D-aspartate
PKA	protein kinase A
PME	pilocarpine model of epilepsy

pMEA	perforated multi-electrode array
popspike	population spike
PrS	presubiculum
s.c.	subcutaneous
SEM	standard error of mean
Sub	subiculum
TLE	temporal lobe epilepsy

1 Abstract

Experimental models and previous studies suggest that seizures are accompanied by disturbances in the β-adrenergic (β-AR) system of the brain. Norepinephrine acting via β-ARs plays a major role in hippocampal plasticity and hippocampus-dependent learning and memory. To elucidate seizure-associated alterations in the norepinephrine-dependent encoding of hippocampal output, the present study investigates the functional consequences of the β-AR mediated synaptic plasticity at CA1-subiculum synapses for the transduction of hippocampal output to the parahippocampal region in an animal model of epilepsy.

Using combined electrophysiological (single-cell and multi-electrode array recordings) approaches, we show that activation of β-AR induces a cell-specific form of long-term potentiation in subicular pyramidal cells that may allow a strengthening of target-specific connectivity to the presubiculum and entorhinal cortex (EC). In pilocarpine-treated animals, the β-AR-mediated modulation of functional connectivity between the hippocampus and distinct parahippocampal target str uctures is disturbed. The attenuated long-term potentiation is associated with a disturbed polysynaptic transmission from the CA1, via the subiculum to the presubiculum, but with a preserved transmission to the medial EC.

The impairment in the β-AR-dependent modulation of information transfer from the hippocampus to its target structures may contribute to hippocampus-dependent deficits like memory impairments and mood disorders which are often observed in patients with temporal lobe epilepsy.

2 Zusammenfassung

Experimentelle Modelle und aktuelle Studien legen nahe, dass epileptische Anflle mit Strungen des adrenergen Systems im Gehirns einhergehen. Noradrenalin, welches an β-adrenerge Rezeptoren bindet, ist fr hippokampale Plastizitt sowie fr das hippokampale Lernen und Gedchtnis von groer Bedeutung. Die vorliegende Arbeit untersucht epilepsieinduzierte Vernderungen der noradrenergen Steuerung von hippokampalen Ausgangssignalen. Gezielt werden die funktionellen Konsequenzen synaptischer Plastizitt, hervorgerufen durch β-adrenerge Rezeptoraktivierung an CA1-Subiculum Synapsen, fr die neuronale Signaltransduktion zwischen Hippocampus und parahippokampal Regionen in einem Tiermodell fr Epilepsie untersucht.

Wir kombinieren elektrophysiologische Methoden (Single-Cell- und Multi-Elektroden-Array Ableitungen) um zu zeigen, dass die Aktivierung von β-adrenergen Rezeptoren eine zellspezifische Form der Langzeit-Potenzierung in subiculren Pyramidenzellen induziert und eine Verstrkung der Konnektivitt zwischen Subiculum und Presubiculum, beziehungsweise Subiculum und entorhinalen Cortex nach sich zieht.

Bei Tieren, die mit dem Parasympathomimetikum Pilocarpin behandelten wurden, ist die β-adrenerge Modulation zwischen dem Hippocampus und verschiedenen parahippokampal Zielstrukturen beeintrchtigt. Die gestrte polysynaptische Transmission zwischen CA1, dem Subiculum und parahippokampalen Zielstrukturen resultiert in einer Abnahme der Langzeit-Potenzierung im Presubiculum, wohingegen die Transmission zum medialen EC intakt bleibt. Diese Beeintrchtigung der β-Adrenorezeptor abhngigen Modulation der Informationsbertragung vom Hippocampus zu seine Zielstrukturen knnen zu hippocampalen Defiziten, wie Gedchtnis- und Stimmungsstrungen beitragen, die hufig bei Patienten mit Temporallappen-Epilepsie beobachtet werden.

3 Introduction

3.1 The Hippocampus and its Role in Learning and Memory

How memories are formed and retrieved is one of the central questions in neuroscience. Previous neurobiological studies began to uncover cognitive properties and neuronal coding underlying our ability to remember every day experiences (episodic memory) and factual knowledge (semantic memory). The mechanism of simultaneous memory consolidation and memory recall depends on bidirectional connections between the neocortex, the parahippocampal region and the hippocampus. In this context, especially the hippocampus appears to play a major role for declarative memory[1] since this brain region processes sensory input and relays this input to a variety of cortical and subcortical brain regions. Thus the hippocampus and notably the subiculum, as a subregion of the hippocampal formation, serve as a major relay station for incoming and outgoing information.

The role of the hippocampus in learning and memory was convincingly demonstrated by the report of Scoville and Millner [1], describing the effects of bilateral medial temporal lobe resection on memory function and thereby linking this brain region intrinsically to memory, independent of other cognitive functions.

Structurally the hippocampus is divided into the dentate gyrus, the cornu ammonis (CA3 and CA1) and the subiculum (see Figure 1). The subiculum, as the most inferior part of the hippocampal formation, receives input from CA1 and entorhinal cortical pyramidal neurons and acts as the main output of the hippocampus. Recent work in rats and humans has demonstrated that the subiculum is critically involved in encoding and retrieval of learned information, underpinning its role in hippocampal-cortical interac-

[1]Declarative memory includes memories which can be consciously recalled and can therefore be classified as episodic memory (autobiographical events) and semantic memory (factual knowledge).

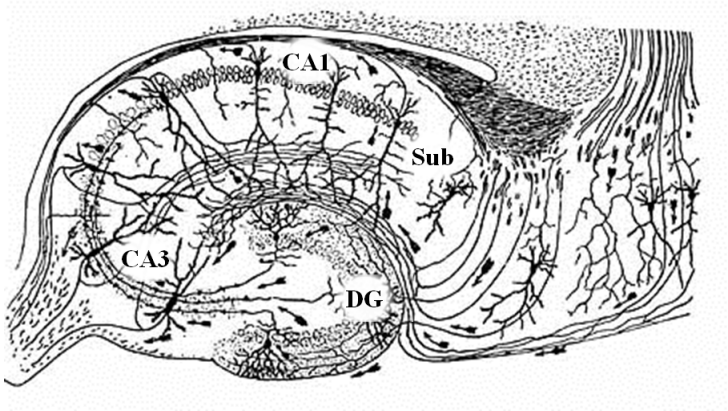

Figure 1: *Drawing of the rodent hippocampus and its neuronal circuitry by Santiago Ramn y Cajal (Histologie du Systeme Nerveux de l'Homme et des Vertebretes, Vol. 1 and 2. A. Maloine. Paris. 1911).*

tion [2]. In rodents and humans, subicular pyramidal cells are classified as either regular- (RS) or burst-spiking (BS) neurons [3, 4]. The distinct spatial distribution of bursting and regular firing cells in the proximo-to-distal and deep-to-superficial axes of the subiculum [5, 6] and the topography of subicular efferents [7, 8, 9] suggest that bursting and regular-spiking cells may target different brain structures in rodents [10, 11]. In particular, the subiculum is connected bi-directionally with the entorhinal cortex (EC) and the presubiculum and there is evidence that subicular RS and BS cells project either to the EC or to the presubiculum, respectively [12, 13].

It was also the hippocampal region where long-term potentiation (LTP) was discovered approximately 40 years ago [14] and still today LTP is probably the most commonly accepted model for learning and memory formation in the nervous system. LTP is characterized by an activity-dependent modification of synaptic efficiency that causes an enduring increase in synaptic strength [15]. This synaptic potentiation occurs within seconds and persists in the range of hours in *in vitro* preparations or even days in freely moving animals.

LTP can moreover be induced in a number of ways and the probably most studied one is LTP induction by activation of the N-methyl-D-aspartate (NMDA) receptor complex, by applying a tetanus[2] to the pathway of interest. Applied to CA1-subiculum synapses, a tetanus triggers two different forms of LTP in BS and RS neurons [16].

LTP in RS cells is dependent on postsynaptic depolarization induced by activation of the glutamatergic α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid (AMPA) receptors. In general, this depolarization activates postsynaptic NMDA receptors, causing an influx of Ca^{2+}(see Figure 2A). Ca^{2+}subsequently binds to respective binding proteins (CaMKII), triggering a signaling cascade that causes insertion of new AMPA receptors into the cell membrane. A prolonged Ca^{2+}influx results in new gene transcription and mRNA translation and subsequently causes an increase in the number of AMPA receptors, which is accompanied by an increase of synaptic connections [17].

In BS neurons on the other hand, LTP also depends on the activation of presynaptic NMDA receptors and postsynaptic depolarization or postsynaptic Ca^{2+} signaling is not required [16]. LTP in BS cells is induced by a presynaptic increase in Ca^{2+}levels which initiate a cAMP-PKA dependent signaling cascade and increase thereby the release probability of synaptic vesicles (see Figure 2B).

[2]Tetanic stimulation is a stimulation pattern composed of four electrical stimuli of 100 Hz for one second with an interstimulus intervall of 10 s.

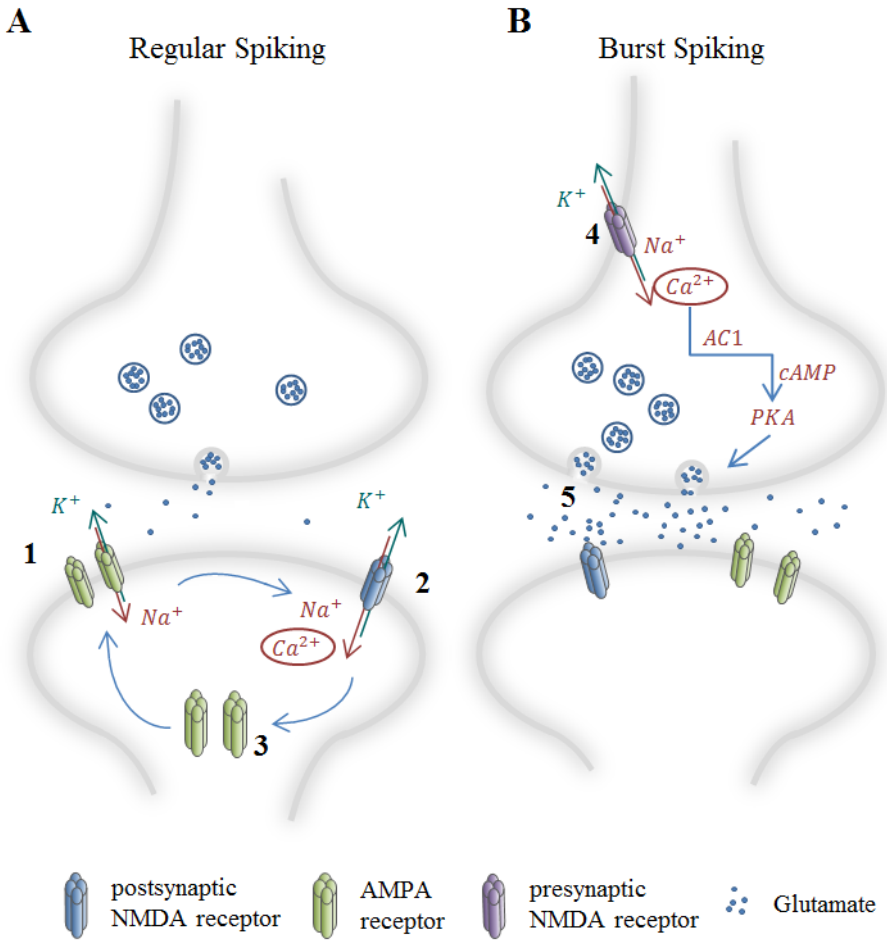

Figure 2: *Subicular RS und BS neurons reveal two different types of LTP at the CA1-subiculum synapses. LTP in RS neurons is induced by AMPA receptor dependent depolarization (1) of the postsynaptic cell by sodium influx and postsynaptic Ca^{2+} increase trough activation of postsynaptic NMDA receptors (2). A persistent Ca^{2+} influx results in new gene transcription and mRNA translation, which increase the number of postsynaptic AMPA receptors at the postsynaptic membrane (3). In BS neurons LTP induction depends on activation of presynaptic NMDA receptors (4), initiating a Ca^{2+} and cAMP-PKA dependent signaling cascade and enhancing vesicular release probabilities (5).*

3.2 Noradrenergic Modulation of Synaptic Plasticity in the Hippocampus

One of the most prominent neuromodulators regarding hippocampal plasticity is norepinephrine (NE)[3], since the hippocampus recieves a strong adrenergic input from the locus coreleus [18, 19, 20]. In response to novel stimuli or emotional arousal, the locus coeruleus is activated. Activation of the locus coeruleus in turn leads to the release of NE in the hippocampus, resulting in an enhancement of synaptic efficiency. NE then acts via β-adrenergic receptors (β-AR), which are highly expressed in the hippocampus and especially in the subiculum [21, 22]. These β-ARs can be subdivided in β1-, β2- and β3-ARs [23] and account for one-third of the adrenergic G protein-coupled receptor family [21]. Once NE is bound to β-ARs, a G-protein-dependent signaling cascade causes an increase of 3',5'-cyclic AMP (cAMP) levels and subsequently activates protein kinase A. Recent studies showed that activation of β-ARs by application of the β-AR agonist isoproterenol[4] results in facilitated synaptic transmission in CA1 pyramidal cells [24, 25] as well as in subicular principal neurons [26] and hence may cause a facilitation of long-term memory storage in the hippocampal network. Blockage of adrenergic receptors in contrast restrains memory retrieval [27, 28].

3.3 Memory Impairments in Temporal Lobe Epilepsy

Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy in humans [29]. It evolves in one or both temporal lobes of the brain and arises either in the medial or lateral part of the temporal structure [29]. TLE arising in the medial part of the temporal lobe affects the hippocampus, parahippocampal structures and the amygdala.

Recent studies revealed that 50-60% of patients suffering from TLE ex-

[3]Norepinephrine is also referred to as noradreneline. In this study the term norepinephrine will be used.

[4]Isoproterenol is a synthetic NE-derivate and a non-selective beta-adrenergic agonist. It's structure is similar to adrenaline.

hibit impairments in episodic memory (time and context dependent memory) but less in semantic memory [30]. These cognitive deficits are an intricated interaction of immutable static factors versus more dynamic and reversible influences. TLE involves irreversible structural lesions on the one hand and dynamic epileptic activity and seizures on the other side. These dynamic factors can have irreversible damaging effects on the temporal lobe structure and mesial temporal sclerosis (also hippocampal sclerosis [5]) has always been a neuropathological main feature of TLE and might contribute to severe hippocampus-dependent memory impairments and mood disorders in patients suffering TLE [32, 33].

3.4 Aims of this Study

Experimental models and studies in humans suggest that seizures are accompanied by disturbances in the adrenergic system [34, 35, 36]. Activation of β-AR by NE triggers signaling mechanisms which are crucial for modulation of synaptic plasticity. In this study we investigate synaptic plasticity at the cellular level and its effect on information processing at the network level. We show that activation of β-AR at CA1-subiculum synapses induces a cell-specific form of LTP in burst- but not in regular-spiking cells. This cell-specific LTP strengthens hippocampal output to the parahippocampal region in a target-specific manner. Based on this findings, we studied alterations of the β-AR-dependent information transfer from the hippocampus to its parahippocampal target structures in control and pilocarpine-treated animals, a model of temporal lobe epilepsy. Using the pilocarpine model of epilepsy, this study examines seizure associated changes in hippocampal synaptic plasticity and its implications on the transfer of hippocampal output to its parahippocampal target structures.

[5] Hippocampal sclerosis or mesial temporal sclerosis is defined by a decrease in neuronal density and cell death especially in hippocampal regions as CA1 and the subiculum [31].

4 Materials and Methods

To investigate the characteristics of functional connectivity between CA1, the subiculum and its target-structures in the in vitro slice preparation, two electrophysiological techniques were used. Single cell recordings were combined with multi-electrode recordings, picturing connectivity to hippocampal target regions.

β-AR dependent LTP was studied by application of the β-AR agonist isoproterenol. Changes in subicular synaptic plasticity and its effect on hippocampal output were observed in several hippocampal target regions under control conditions. Based on this work, the pilocarpine model of epilepsy was employed to investigate seizure associated modifications of the information transfer from the hippocampus to its parahippocampal target structures in epileptic animals.

4.1 Pharmacological Tools

The following drugs were used in the present study among others (see Table 1): (-)- bicuculline methiodide (5 M), isoproterenol (10 M). Substances were dissolved and stored as stock solutions at 1000 times the end concentration in distilled water.

reagent	source
(-)-Bicuculline methiodide	Abcam, biochemicals, UK
Diazepam	Ratiopharm, Germany
Glucose	Sigma-Aldrich, Germany
()-Isoproterenol	Sigma-Aldrich, Germany
(-)-Methylscorpolamine bromide	Sigma-Aldrich, Germany
Pilocarpine hydrochloride	Sigma-Aldrich, Germany
Saccharose	Merck, Germany
Saline 0.9%	Fresenius Kabi, Germany

Table 1: *Employed chemicals and source of supply.*

4.2 Media Composition

Solutions and compounds used for slice preparation, tissue storage and recordings are summarized in Table 2. Previous work has found that increasing the concentrations of $MgSO_4$ and $CaCl_2$ in artificial cerebrospinal fluid (ACSF) to 4 mM each, prevents the occurrence of polysynaptic responses [37, 38]. This change in ACSF composition did not affect the expression of LTP at CA1-subiculum synapses [16]. Hence this modified physiological ACSF (recording ACSF) was employed in the present study during all measurements. GABA-ergic transmission was blocked using bicuculline (5 M) which was applied throughout the entire course of the experiment and for at least 10 minutes before recording.

solution	components [mM]	solvent and pH
physiological ACSF (for preparation)	$NaCl$ 129, NaH_2PO_4 1.25, $NaHCO_3$ 21, KCl 3, $CaCl_2$ 1.6, $MgSO_4$ 1.8 and *glucose* 10	ddH_2O/ 7.4
saccharose based ACSF	$NaCl$ 87, NaH_2PO_4 1.25, $NaHCO_3$ 26, KCl 2.5, $CaCl_2$ 0.5, $MgCl_2$ 7, saccharose 75 and *glucose* 25	ddH_2O/ 7.4
recording ACSF (for MEA and patch-clamp recordings)	$NaCl$ 129, NaH_2PO_4 1.25, $NaHCO_3$ 21, KCl 3, $CaCl_2$ 4, $MgSO_4$ 4, *glucose* 10 and $(-)-$ *bicucullinemethiodide* 0.005	ddH_2O/ 7.4
intracellular solution	$K-gluconate$ 135, KCl 20, $HEPES$ 10, *phosphocreatine* 7, $Mg-ATP$ 2,$Na-GTP$ 0.3, $EGTA$ 0.2	ddH_2O/7.35

Table 2: *Composition of intra- and extracellular solutions employed for tissue preparation and recording.*

All chemicals used for media composition were purchased from Merck (Germany) or Roth (Germany).

4.3 Tissue Preparation

All experiments were conducted in agreement with international and national guidelines and approved by the local health authority (LAGeSO-Landesamt fr Gesundheit und Soziales Berlin) and the local animal care and use committee (ACUC). Male Wistar rats were decapitated under deep isoflurane anesthesia (3%) and their brains were quickly removed and placed in ice-cold, oxygenated, physiological ACSF. Since it has previously been shown that functional fiber connections between EC and the hippocampus are largely retained in horizontal slice preparations [39], the brains were sliced horizontally (see Figure 3, left) from the ventral-to-dorsal part with a vibratome (Leica VT1200, Leica Microsystems GmbH, Wetzlar, Germany). Slices of 400 m thickness were obtained including the hippocampus, presubiculum and entorhinal cortex (see Figure 3, right). For MEA recordings, slices were preserved in an interface chamber after preparation, constantly perfused with prewarmed, physiological ACSF (34C) and recovered from preparation at least for one hour before measurement. Slices were used for up to six hours after preparation.

For patch clamp recordings, the tissue was prepared in ice-cold, oxygenated saccharose-based ACSF. After preparation the slices were kept under submerged conditions. Initially, they recovered for at least 25 min in saccharose-based ACSF at 34C and were subsequently stored at room temperature in physiological ACSF. Slices were used for up to seven hours after preparation.

All solutions were kept at a pH of 7.4 and continuously aerated (95% O2 and 5% CO2).

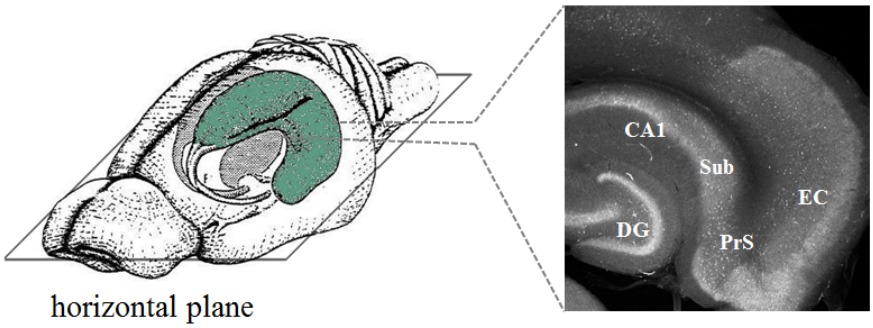

Figure 3: *Scheme of horizontal brain slice orientation (left, [40]) and illustration of an acute brain slice including hippocampus, presubiculum and entorhinal cortex.*

4.4 The Pilocarpine Model of Epilepsy

The pilocarpine model of epilepsy (PME) displays the main characteristics of human temporal lobe epilepsy in rats and mimics hippocampal sclerosis. Administration of the muscarinic agonist pilocarpine[6] causes status epilepticus and induces recurrent, chronic seizures in a long term range. The induction of status epilepticus results in a widespread neuronal cell loss in several brain regions including the hippocampus [41, 42]. Initial damage immediately occurs in the hippocampus within minutes after status epilepticus.

The PME can be divided into three specific periods: status epilepticus, silent period, chronic period [43, 44, 45].

4.4.1 Status Epilepticus

At least one week prior to pilocarpine treatment, male Wistar rats were housed in groups of five, undergoing a standard light-dark cycle. 30 minutes prior to pilocarpine treatment, rats were pretreated with a subcuta-

[6]Pilocarpine is a parasympathomimeticum, originally obtained from the leaves of a tropical South American shrub (Pilocarpus). It acts as a non-selective muscarinic receptor agonist.

neous[7] injection of the muscarinic antagonist methylscopolamine (1 mg/kg) to reduce peripheral cholinergic effects. The animals were subsequently intraperitoneally[8] (i.p.) injected with pilocarpine at a concentration of 340-350 mg/kg, when five to six weeks old (120-200 g).

Immediately after pilocarpine injection, the neuronal background activity in the hippocampus is replaced by a theta rhythm [46]. This theta rhythm triggers a fast spiking activity in the hippocampus that spreads to the cortex and generates ictal events. Within two hours after pilocarpine administration, about 56% of the treated animals show ictal periods every five to ten minutes which finally develop into a convulsive, generalized status epilepticus. Status epilepticus can be described as a condition in which the brain is in a permanent state of abnormal excessive or synchronous neuronal activity [47]. Each animal stayed in status epilepticus for at least 75-90 min after seizure onset, before status epilepticus was terminated by administering diazepam (10 mg/kg i.p.). If not terminated, this pattern of electroencephalographic activity would last for several hours [43, 44, 45, 48, 49].

4.4.2 Silent Period

The behavioral signs of status epilepticus ceased approximately 15-20 minutes after termination of status epilepticus. One side effect of pilocarpine treatment and possibly a result of brain damage during status epilepticus is a significantly elevated level of aggressive behavior [50]. Hence rats were kept separately on a standard light-dark cycle when necessary, where they went through a seizure free, latent period.

The time interval of seizure free, latent periods differed a lot among epileptic rats. Rats developed chronic seizures the earliest about six to eight weeks after pilocarpine treatment, but seizure onsets could also be observed up to six months and more after pilocarpine treatment. In rare cases, rats did not develop chronic seizures, even if they experienced status epilepticus.

[7]Subcutaneous injections are administered into the subcutis.

[8]Intraperitoneal injection denotes the injection of a substance into the body cavity (peritoneum).

4.4.3 Chronic Period

After six to eight weeks, all rats which experienced status epilepticus underwent video observation[9]. Rats (now 400-600 g) were monitored using a CF4/1 video camera (Kappa, Gleichen, Germany) with Digiprotect software (Abus Security, Affing, Germany). Recordings were made for a period not exceeding 72 hours and spontaneous seizures were noted in subsequent video analysis. Only after showing spontaneous seizures, animals were selected for experiments, and were sacrificed between 15 and 50 weeks of age. The signs and symptoms of spontaneous epileptic seizures vary among rats. The most distinct sign of an epileptic condition is the tonic-clonic seizure, characterized by loss of consciousness, collapse with subsequent convulsion and rhythmic jerking of both arms and legs (see Figure 4: left- seizure free, right-tonic-clonic seizure). But seizures can also be expressed more subtle by an acampsia of the tail or by a rat only straightening up.

The incidence of seizures occurring during the chronic period varies substantially between pilocarpine treated rats. While some pilocarpine injected animals showed a low seizure frequency during video observation, others presented relatively regular seizure patterns each day and some presented seizure clusters in short time periods [45, 44, 51, 48, 43]. Age-matched animals underwent the same procedure but were injected with physiological saline and used as controls.

[9]Rats which did not experience status epilepticus were not monitored or included in this study.

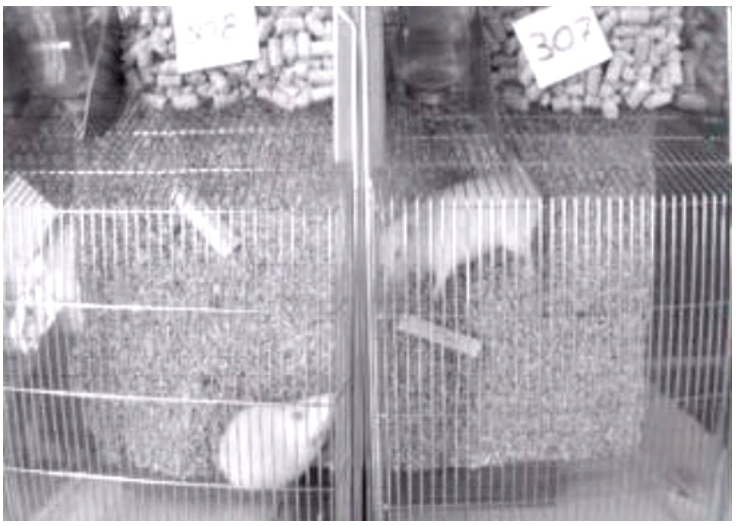

Figure 4: *Representative monitoring of two pilocarpine treated rats. While the animal on the left is showing natural behavior, the rat on the right is experiencing a convulsive epileptic seizure. This condition is characterized by straightening up with a subsequent collapse and stiffness and cramping of arms, legs and tail in an atypical position.*

All experiments were approved by the Regional Berlin Animal Ethics Committee (G 0024/04; G 0391/08) and the local health authority (Landesamt fr Gesundheit und Soziales Berlin) and conducted in line with national and international guidelines.

4.5 Patch-Clamp Recordings

All patch-clamp recordings were performed in collaboration with Kate E. Gilling and Julia C. Bartsch.

Electrophysiological single cell patch-clamp recordings were performed from the subiculum, presubiculum, medial and lateral entorhinal cortex. Cells in the entorhinal cortex were chosen from layer V, as these neurons are believed to be synaptic targets of hippocampal output [52, 5]. Previously published works [53, 54, 55] were used for identifying electrophysiological characteristics and the anatomical location of recorded cells. To characterize membrane properties and investigate cellular discharge behavior, hyper- and

depolarizing current steps were applied in current clamp mode in steps of 50 pA, starting from -250 pA and for a period of 500 ms. Cells were only included in measurements and statistics, if their resting membrane potential was about -55 mV and the series resistance of less than 25 $M\Omega$. To measure series and input resistance throughout voltage-clamp recordings a hyperpolarizing step of of 5 mV was applied for a 300 ms second duration and 600 ms before the evocation of synaptic responses. All recordings of evoked excitatory postsynaptic currents (EPSCs) were done in voltage-clamp mode at a holding potential of -70 mV. The stimulus intensity was set after adjusting the EPSC amplitudes to 4060% of the maximum response by applying an input-output curve (I/O curve). Baseline responses were recorded at 0.1 Hz for at least 10 min. A pair of postsynaptic currents was evoked every 30 s using a pair of 100 s pulses (pulse separation was 50 ms) applied via a self-made unipolar stimulation electrode. When pyramidal cells in the subiculum were recorded, the stimulus was applied to the alveus between the CA1 and subiculum. To prevent epileptiform activity due to the recurrent connections in the subicular network $CaCl_2$ and $MgSO_4$ concentrations of the ACSF were increased to 4 mM [37, 56, 38]. All experiments were performed in the presence of the $GABA_A$ receptor antagonist bicuculline (5 M) to block $GABA_A$ receptor-mediated responses.

Patch-clamp electrodes had a resistance of 4 to 6 $M\Omega$ and were manufactured using a horizontal DMZ Universal Puller (Zeitz Instruments GmbH, Martinsried, Germany). For recordings, electrodes were filled with intracellular solution (see Table 2) and slices were perfused with prewarmed recording ACSF (34C) in a mini-bath chamber (submerged conditions) at a rate of 5-6 ml/min. Isoproterenol was applied at a concentration of 10 M in all experiments. Signals were low-pass filtered at 3 kHz and sampled and processed at 10 kHz. Filter and sampling frequency were chosen applying the Nyquist-theorem, were the sampling rate has to be at least twice the highest analog frequency in order to convert all components of the analog signal into a digital one. All patch-clamp recordings were performed using a Multi-

clamp 600B amplifier in conjunction with a Digidata 1440A interface (both Molecular Devices LLC, Sunnyvale, CA, USA).

4.6 Multi-Electrode Array Recordings

In contrast to the single-cell patch-clamp technique, multi-electrode arrays (MEAs) allow simultaneous recordings of excitatory postsynaptic field potentials (fEPSP) in several regions of acute brain slices (see Figure 5). The MEAs featured 200 m interelectrode spacing and 30 m diameter and the electrode grid had a diagonal of approximately 2.3 mm (see Figure 6).

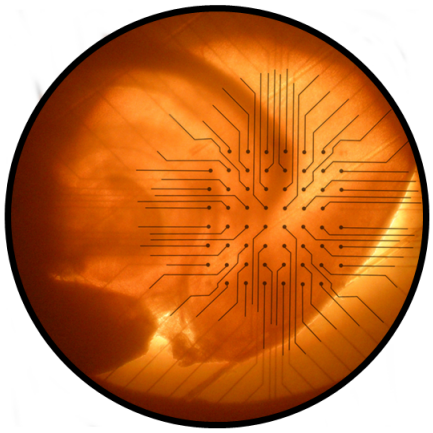

Figure 5: *Horizontal, hippocampal brain slice on a MEA grid, visualized under a light microscope (MEA electrodes are highlighted).*

Neural network activity recordings were performed with a MEA1060-UP-BC amplifier (Multichannel Systems, Reutlingen, Germany) with 60 channels in which neuronal activity was digitized with a sampling rate of 25 kHz. Online data acquisition was performed with MC_Rack software, provided by Multichannel Systems.

Excitatory postsynaptic field potentials were evoked in the alveus every 10 s using 100 s pulses via an external stimulation electrode. When alternat-

ing stimuli were applied, an inter-pulse interval of 15 s was chosen between each stimulus. Based on an input-output curve (I/O curve), the amplitudes of evoked fEPSPs and EPSCs were adjusted to approximately 40-60% of the maximum synaptic response. All experiments were performed in the presence of the $GABA_A$ receptor antagonist bicuculline (5 M) to block $GABA_A$ receptor-mediated responses.

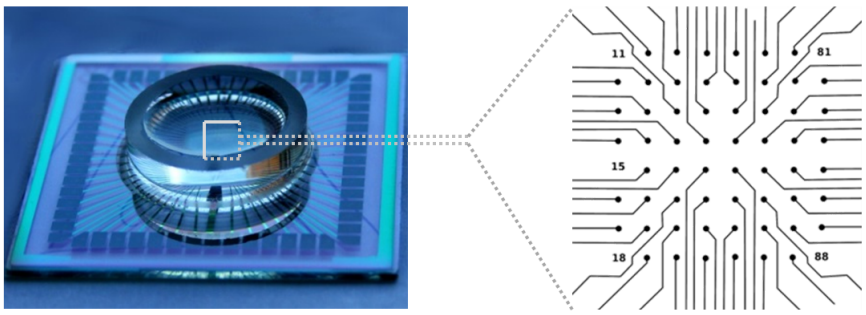

Figure 6: *Employed MEA device (glass) with 59 titanium nitride recording electrodes. Scheme on the right is a blow up of the electrode grid and illustrates the arrangement of all the electrodes.*

4.7 Data Analysis

4.7.1 Software

Patch-Clamp recordings were performed using PClamp software. Analysis of the recordings was performed using Clampfit software (both Molecular Devices).

For MEA recordings MC_Rack software (Multichannel Systems, Reutlingen, Germany) was employed for online data acquisition. Stimulation protocols were written and applied via MC_Stimulus software (Multichannel Systems, Reutlingen, Germany) and by using an external stimulation electrode.

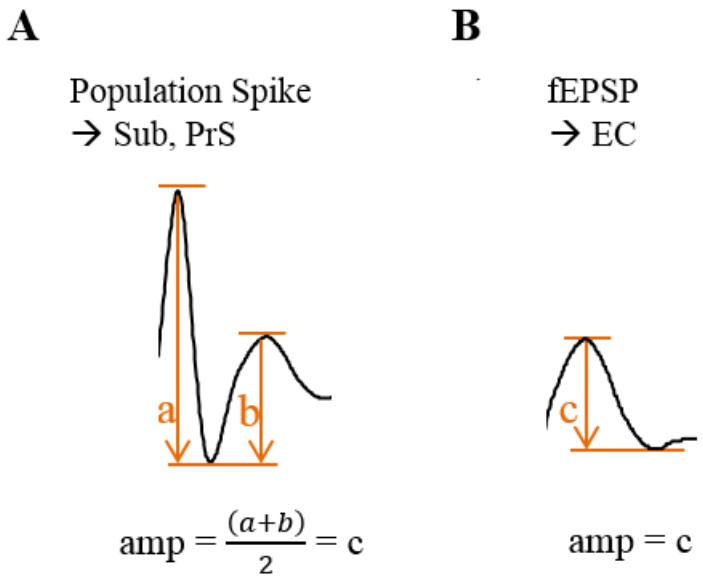

Figure 7: *Evaluation of field amplitudes depended on the signalling shape. (A) Subicular popspike amplitudes were determined by applying linear interpolation. (B) For fEPSPs of the EC for example, the distance from the normalized baseline to the maximum of the trace was defined as amplitude.*

MEA data was analyzed by a homemade matlab (MathWorks, Natick, MA, USA) script written in collaboration with Jan-Oliver Hollnagel. For analysis, the MEA raw data of each channel and for each stimulus were loaded and superposed. Signals were filtered by using a lowpass butterworth filter (sixth order). Signalling amplitudes for each trace were calculated according to their shape. While population spikes, only arising in the subiculum and presubiculum, were analyzed via linear interpolation, fEPSPs amplitudes were used for analysis of the entorhinal cortical region as described in Figure 7.

Three dimensional plots of representative MEA recordings were generated employing a modified version of MEA-Tools (Ulrich Egert, University of Freiburg, Germany, [57]). Within those 3D plots, x- and y-axis represent the 8x8 electrode grid, while the Z-projection displays the relative increase

of normalized fEPSPs compared to baseline level.

To summarize, plot and illustrate the results, Microsoft Office Excel 2010, Microsoft Office Power Point 2010 (Microsoft Corporation, Redmond, WA, USA), Graph Pad Prism (Graph Pad Software, Inc., La Jolla, CA, USA), gimp (GNU image manipulation program) and ImageJ (National Institutes of Health, Bethesda, MA, USA) were used respectively.

4.7.2 Statistics

To assess the magnitude of LTP, the final 10 min of wash out of each measurement were averaged and related to the amplitudes of 10 min of stable baseline recordings.

Graphs and figures show mean values with respective standard errors of mean (SEM). Where appropriate, a Student's t-test was performed in order to exert pair-wise statistical comparison for paired and unpaired samples. Significance level was set to $p<0.05$ (*), $p<0.01$ (**).

5 Results

5.1 Effect of β-AR Activation in the Subicular Region of Control and Pilocarpine-Treated Rats

The subiculum is located between the hippocampal area CA1 and the entorhinal cortex and is a major output structure of the hippocampal formation. It relays information from CA1, the EC and the midline thalamic nuclei [58] via projections to various cortical and subcortical regions [59, 60, 5].

To study how information is transferred from CA1-subiculum synapses, via the subiculum to parahippocampal target structures, we first characterized subicular pyramidal neurons. To analyze characteristic properties of subicular neurons on a single cell level, such as membrane properties and spiking behavior, we performed patch-clamp recordings (see scheme Figure 8A) in control and pilocarpine-treated animals.

Single cell recordings were accompanied by MEA recordings to determine subicular network properties along the proximo-to-distal axis in both animal groups.

5.1.1 Synaptic and Membrane Properties of Subicular Pyramidal Cells during β-AR Activation

All patch-clamp experiments were performed in collaboration with Kate E. Gilling and Julia C. Bartsch.

Subicular pyramidal neurons can be divided into at least two groups based on their spiking properties: regular- (RS) and burst-spiking neurons (BS) [3, 8, 61, 7]. To investigate synaptic and membrane properties of both neuronal types, an external stimulation electrode was placed in proximity to the CA1-subiculum synapses, while synaptic responses were recorded in subicular pyramidal cells (Figure 8A).

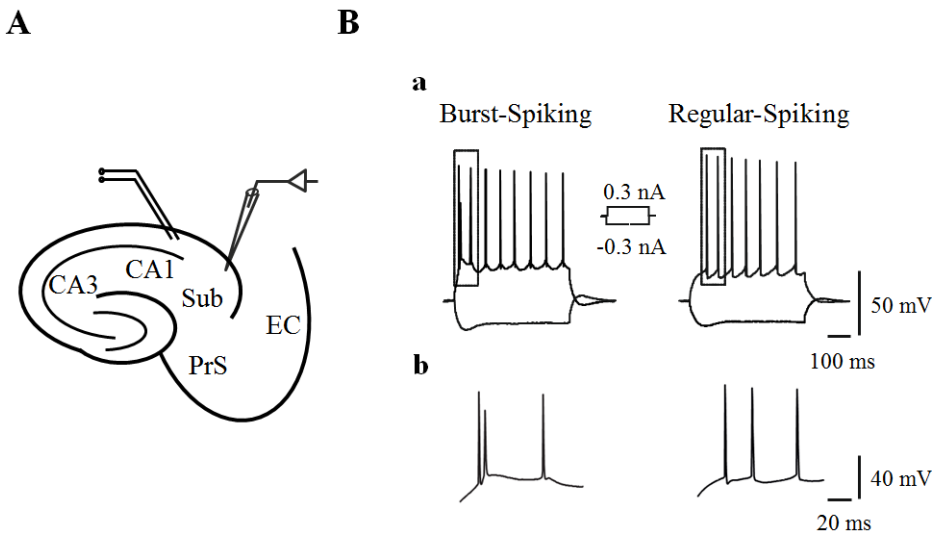

Figure 8: *(A) Scheme of stimulation and recording sites for single-cell patch-clamp recordings in a horizontal hippocampal brain slice. Hippocampal areas: CA3, CA1; Sub: subiculum; PrS: presubiculum; EC: entorhinal cortex; (B) Discharge behavior of subicular BS and RS neurons to depolarizing and hyperpolarizing current steps. BS cells display single burst discharges followed by a train of single spikes at stronger current pulses. RS cells discharge with single spikes or with single spike trains at stronger current pulses. Note the different initial spike frequencies in each cell type.*

Subicular BS cells, which were preferentially recorded in the distal subiculum, responded upon brief current injection with a burst of two to four action potentials at high frequency. RS cells in contrast fire a train of single action potential. In response to stronger current pulses RS cells show trains of single action potentials, whereas BS cells respond with one or more high-frequency bursts, usually followed by regular spiking (Figure 8B) [62, 9].

Previous work has shown that activation of β-adrenoceptors by isoproterenol has transient effects on membrane potential and input resistance of both regular and burst-firing neurons in the subiculum of young rats (4-6 weeks) [26]. Similar effects were seen in the present work which employs older rats (30-52 weeks). Consistent with previous studies, application of

the β-AR agonist isoproterenol resulted in a transient decrease of input resistance, which returned to baseline level after wash out. This effect was similarly proven in BS and RS neurons of both pilocarpine-treated and control animals (maximum effects; control BS: 35.57.8%, $p<0.01$, n=9; control RS: 61.917.5%, $p<0.05$, n=6; pilocarpine BS: 30.24.4%, $p<0.01$, n=8; pilocarpine RS: 42.438.5%, $p<0.05$, n=5; Figure 9A and B). In both animal groups the decrease in input resistance was accompanied by transient depolarization currents of BS and RS cells, as indicated by an increase in the negative current required to hold cells at -70mV. The isoproterenol-induced depolarization differed neither significantly between RS and BS neurons, nor between control and pilocarpine-treated animals (maximum effects; control BS: 23.910.4%, $p<0.01$, n=9; control RS: 42.416.1%, $p<0.01$, n=6 ; pilocarpine BS: 17.84.3%, $p<0.01$, n=8; pilocarpine RS: 42.916.3%, $p<0.05$, n=8; Figure 9C and D). After wash out of isoproterenol, depolarization reversed within 10 min.

Figure 10 shows absolute baseline values of holding current (Ihold in pA), input resistance (Rin in M) and EPSC amplitudes (in pA) of RS and BS cells in control and pilocarpine-treated animals. Between control and pilocarpine-treated animals, we found no significant difference in Ihold, Rin and EPSC amplitude of RS or BS cells, respectively.

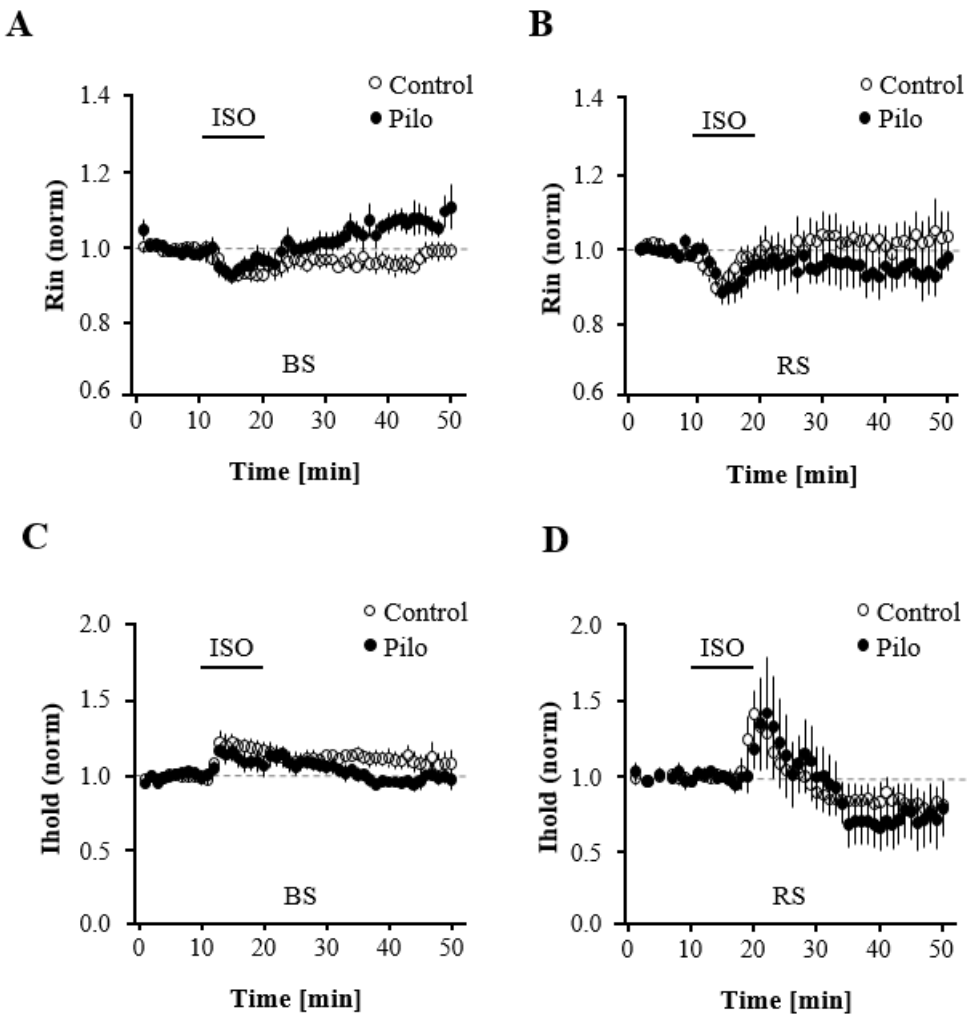

Figure 9: *(A,B) Isoproterenol application results in a reduction of the input resistance of BS and RS cells in epileptic and control animals. Averaged time course of input resistance before, during and after isoproterenol application. (C,D) Isoproterenol application depolarizes BS and RS cells in both animal groups. Averaged time course of the holding current before, during and after isoproterenol application.*

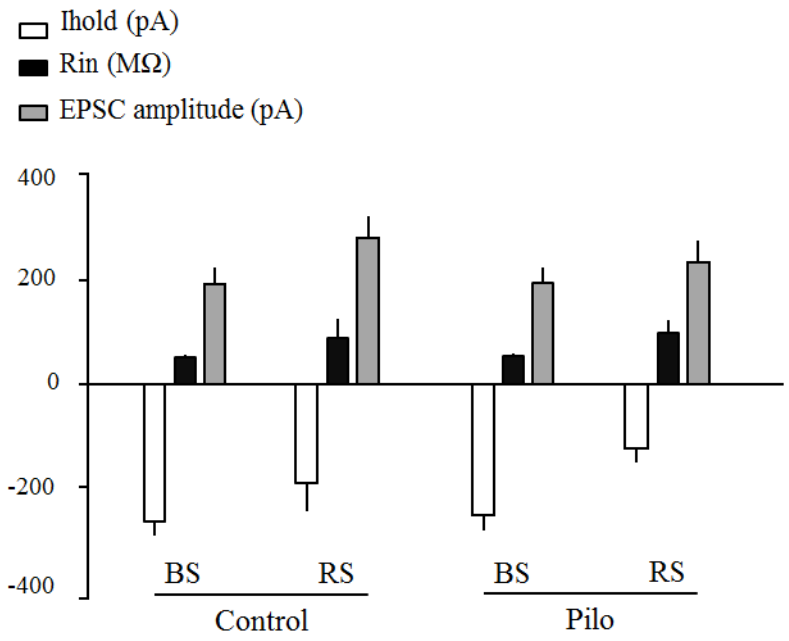

Figure 10: *Absolute baseline values of holding current (Ihold in pA), input resistance (Rin in M) and EPSC amplitudes (in pA) of RS and BS cells in control and pilocarpine-treated animals.*

5.1.2 Absence of Isoproterenol-Induced LTP in RS Cells

A distinct difference between BS and RS neurons is the ability of BS neurons to express LTP upon β-ARs activation. As recently described [26], application of the β-AR agonist isoproterenol for 10 min induces a stable, chemical LTP of evoked EPSCs in BS cells (control BS: 44.111.9%, $p<0.01$, n=7; Figure 11A). The maximal increase of EPSC amplitudes was reached within 10 minutes after isoproterenol application and persisted for at least 40 minutes. RS neurons were transiently depolarized by isoproterenol application and EPSC amplitudes returned to baseline level after wash out. Thus, LTP was completely absent in RS neurons (control RS: 5.97.2%, p=n.s., n=5; Figure 11B).

Results obtained from pilocarpine treated animals were not significantly different compared to control preparations. As in control animals, BS neurons revealed a stable LTP upon β-AR activation, while LTP was completely absent in RS neurons (pilocarpine BS: 58.323.8%, $p<0.05$, n=7, Figure 11A; pilocarpine RS: 10.78.6%, p=n.s, n=6, Figure 11B).

Thus the relative increase of EPSC amplitudes through β-AR activation shows the same characteristics in pilocarpine-treated animals and controls in BS and RS neurons. These results indicate that the mechanism of LTP induction in subicular BS neurons is not disturbed in pilocarpine treated animals.

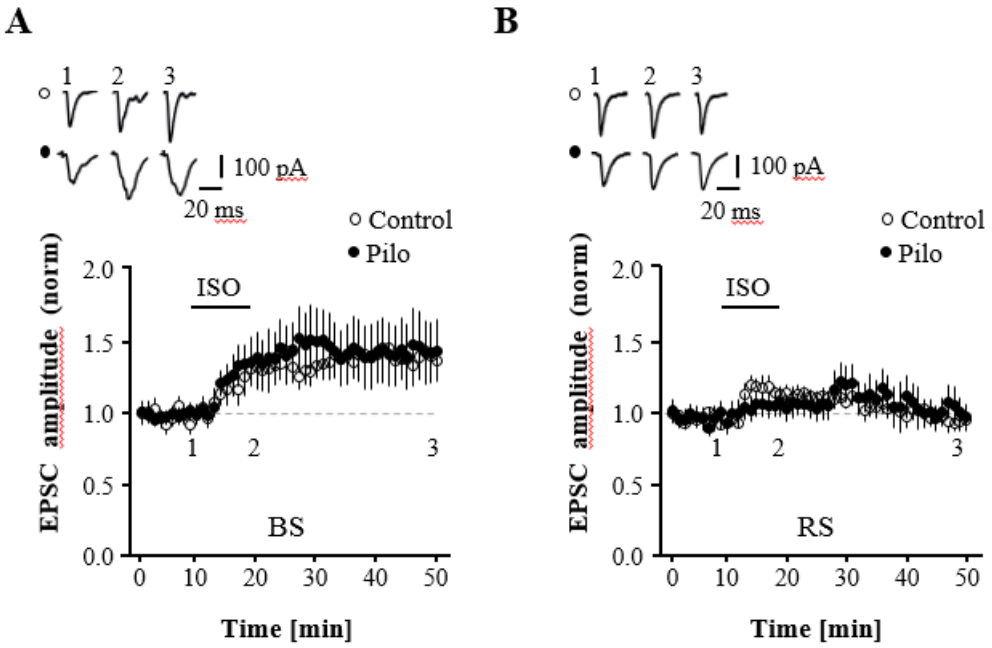

Figure 11: *(A, B) Application of the β-adrenergic agonist isoproterenol (10 μM) induces chemical LTP in BS neurons of control and pilocarpine-treated rats. RS neurons of both animal groups are not affected. Averaged time courses of evoked EPSC amplitudes are shown before, during and after isoproterenol application.*

5.1.3 Region-Specific Decline of Subicular Network Plasticity in Pilocarpine-Treated Rats

Within the subicular region the two distinct types of pyramidal cells are distributed differently along the proximo-to-distal axis. The number of BS neurons increases along the proximo-to-distal axis, while the number of RS neurons decreases [62, 13, 9]. Hence, there are more BS neurons in proximity to the presubiculum (distal) and more RS cells close to CA1 (proximal) as shown in figure 12. Based on these findings, we hypothesized that the increase in synaptic efficiency after β-AR activation is not evenly distributed within the subiculum, but has a stronger prevalence towards the distal end. We expected a pronounced LTP in proximity to the presubiculum (high number of BS neurons) since β-AR dependent LTP is restricted to BS neurons.

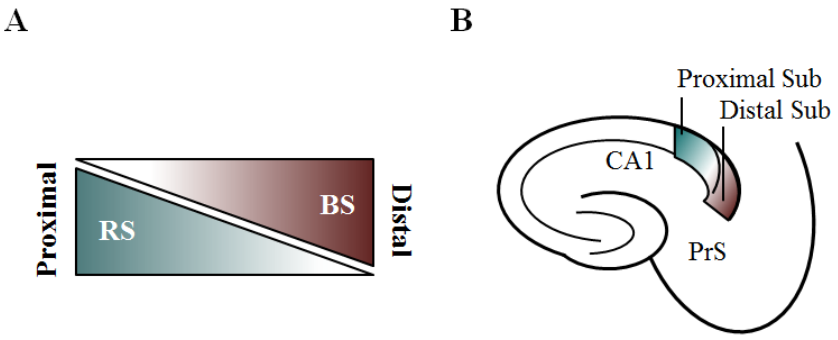

Figure 12: *RS and BS neurons are distributed in an organized fashion along the proximo-to-distal axis. (A, B) The density of BS neurons increases towards the presubiculum, while the number of RS cells increases in proximity to CA1. (Scheme based on Kim and Spruston, 2012 [13])*

To examine subicular LTP at the network level, we employed a multi-electrode array which allows a high spatial resolution and the detection of peak-responses in a neuronal network. Hippocampal brain slices were placed on the MEA grid as shown in Figure 13A. Single, paired pulse stimuli were applied via a stimulation electrode at a low frequency (0.1 Hz) to subicular afferents to evoke fEPSPs in the subiculum. Before isoproterenol application,

a stable baseline was recorded for at least 10 min (Figure 13B, left column). Isoproterenol (10 M) was consequently applied for 20 min and washed out for 30 min.

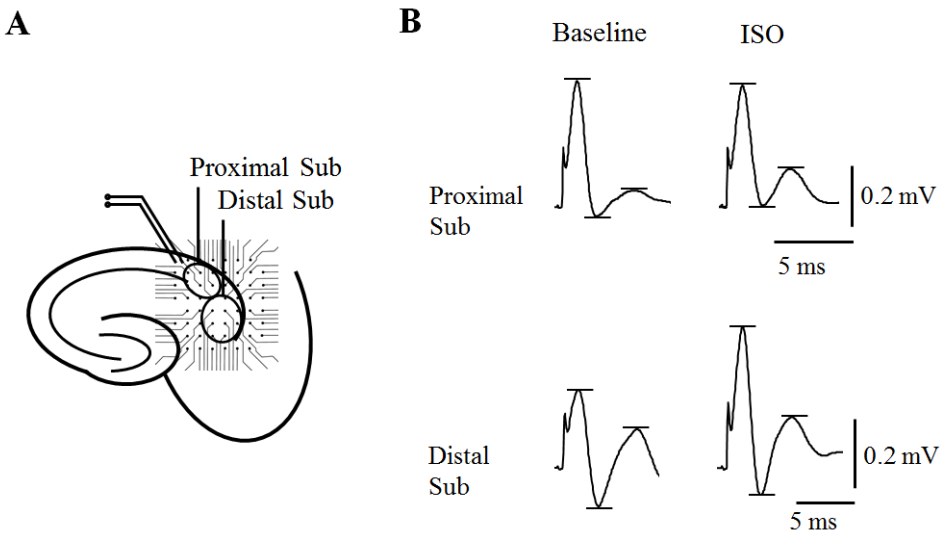

Figure 13: *(A) Positioning of the hippocampal brain slice on the MEA electrode grid and location of the stimulation electrode. (B) Characteristic fEPSPs of the proximal and distal subiculum of control animals before and after isoproterenol application (10 μM).*

Prior to each measurement, an input-output curve (I/O curve) was generated to get a correlation between stimulation strength and synaptic response in general and to adjust fEPSPs to approximately 50% of the maximum synaptic response. Comparing the I/O curves obtained from pilocarpine-treated and control animals indicated a significantly increased slope in pilocarpine treated animals (slope control proximal: 0.180.005, n=6, pilocarpine proximal: 1.150.067, $p<0.01$, n=7; Figure 14A; slope control distal: 0.270.007, n=6, pilocarpine distal: 0.780.036, $p<0.01$, n=7, Figure 14B). The average fEPSPs were about two to three folds stronger in magnitude in pilocarpine-treated rats compared to the fEPSP amplitudes of control animals. These results indicate that epileptic tissue exhibits a higher neuronal excitability.

Application of isoproterenol caused a stable LTP that persisted for at least 50 min in control animals (see Figure 13B). As expected, fEPSPs were facilitated in the distal part of the subiculum (high fraction of BS cells) compared to the proximal subiculum (low fraction of BS cells). LTP in the two subregions differed by a factor of four (fEPSP increase; control proximal: 22.17.4%, $p<0.01$, n=9, Figure 14C; control distal: 92.216.3%, $p<0.01$; n=9, Fig. 14D). In contrast, the isoproterenol-induced increase of fEPSPs was significantly smaller in the two subicular regions of pilocarpine-treated rats. The most prominent decline of LTP was found in the distal part of the subiculum (fEPSP increase; pilocarpine proximal: 8.73.6%, $p<0.01$, n=7, Figure 14C; pilocarpine distal: 29.45.6%, $p<0.01$, n=7, Figure 14D). Interestingly, the absolute magnitude of fEPSP amplitudes were enhanced in pilocarpine animals (fEPSP amplitude baseline/ wash out; control proximal: 36.47.0V/ 44.57.5V, n=6; control distal: 106.044.5V/ 202.342.7V, n=7; pilocarpine proximal: 90.430.7V/ 98.332V, n=7; pilocarpine distal: 228.468.2/ 295.6 70.3V, n=7, Figure not shown). On average, the amplitudes of subicular baseline responses were more than doubled in pilocarpine treated rats which indicates a higher excitability compared to control animals (Figure 14A and B).

Hence there is a stronger change of fEPSP amplitudes during isoproterenol application in control animals though the magnitude of the amplitudes is enhanced in pilocarpine treated rats. These results suggest that, though isoproterenol-induced LTP in BS cells is not disrupted in pilocarpine-treated rats, the network plasticity is indeed impaired in epileptic animals.

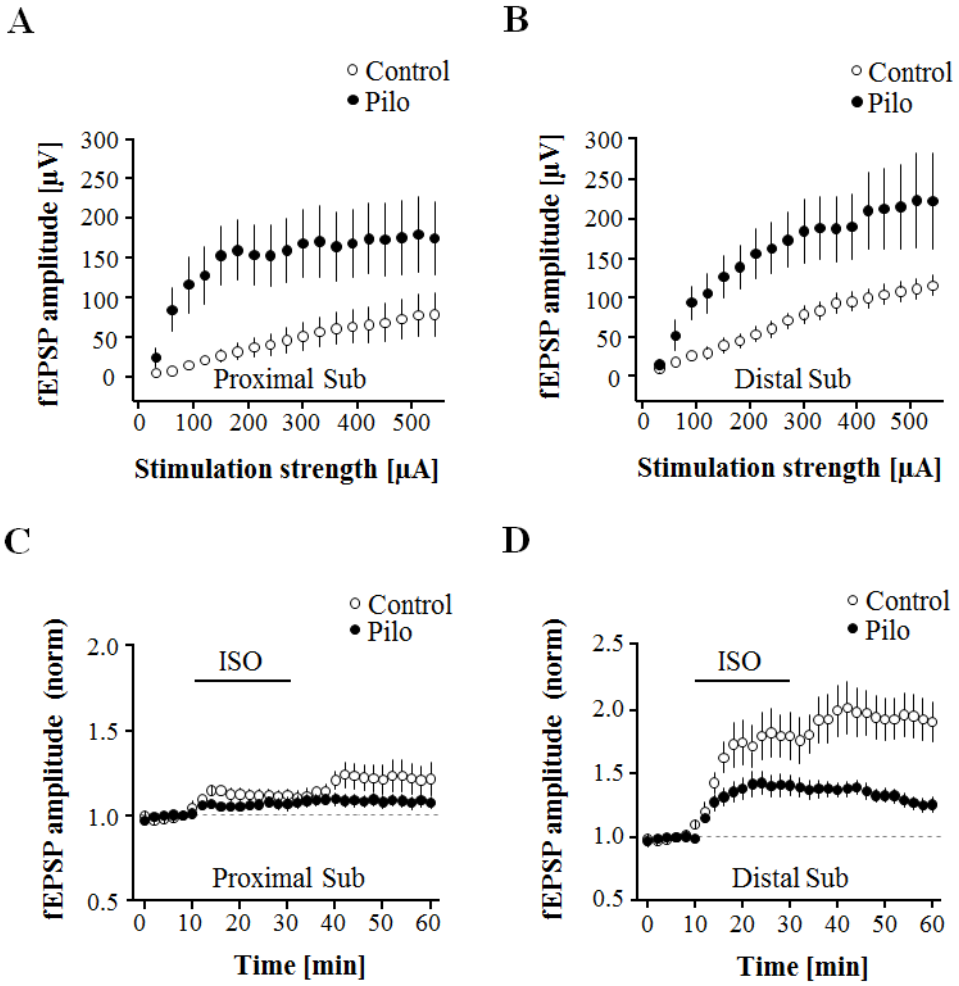

Figure 14: *(A, B) Input-output curves recorded in the subicular region of control and pilocarpine treated animals.(C, D) Average time courses of normalized fEPSPs in the proximal and distal subiculum of control and pilocarpine treated animals after application of isoproterenol. Note the substantial decline of LTP in the distal subiculum of pilocarpine-treated animals.*

5.2 Effect of β-AR Activation on the Connectivity between CA1, Subiculum and Parahippocampal Target Regions

Next we studied isoproterenol-induced LTP at CA1-subiculum synapses and it's consequences for synaptic transmission from CA1 via the subiculum to the presubiculum (PrS) and the entorhinal cortex (EC). To detect parahippocampal target structures and identify pathways of the signal processing between subiculum and parahippocampal regions, we first employed the MEA device.

5.2.1 β-AR-Dependent Alterations in the Connectivity between CA1, Subiculum and Parahippocampal Regions in Pilocarpine-Treated Rats

To stimulate subicular pyramidal cells, the electrode was placed in the dendritic layer at CA1-subiculum synapses as shown in figure 15A. Subsequently, submaximal fEPSPs were recorded from 59 planar electrodes located in the distal part of the subiculum, the presubiculum and deep layers of the medial EC (sample traces shown in Figure 15B). We aimed to assess if LTP, induced in the subiculum by β-AR activation, affects the information transfer from CA1 through the subiculum to its parahippocampal target structures and whether this mechanism would be disturbed in pilocarpine-treated animals.

To determine the relationship between the biological response and the electrical stimulation we also analyzed input-output curves for the presubiculum and the mEC. The I/O curve of the presubiculum indicated a decreased neuronal excitability. Accordingly, our results suggest target specific disturbances in β-AR-mediated polysynaptic transmission between the hippocampus and its parahippocampal target structures in pilocarpine-treated animals.

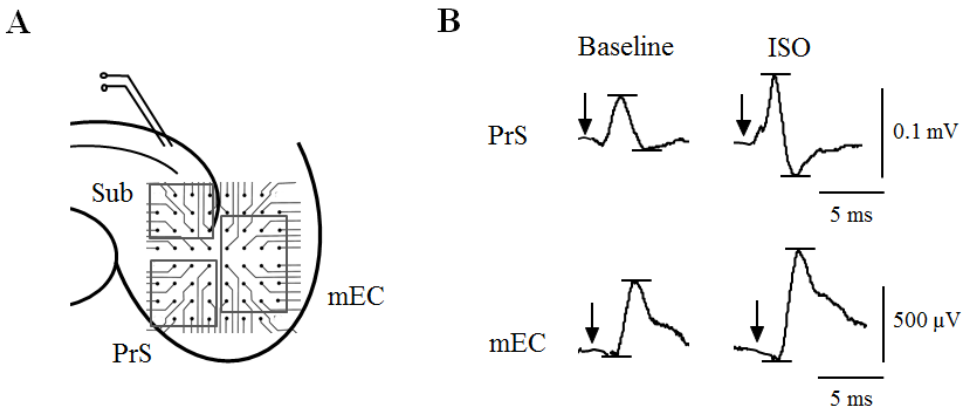

Figure 15: *(A) Schematic illustration of a horizontal hippocampal brain slice and the positioning of the stimulation electrode and the MEA electrode grid. Sub: subiculum, PrS: presubiculum, mEC: medial entorhinal cortex. (B) Representative fEPSPs of the presubiculum and the medial entorhinal cortex before (baseline) and after application of isoproterenol (ISO, 10 µM).*

We found distinct target structures in the parahippocampal region where fEPSPs were facilitated after LTP induction in the subiculum by isoproterenol application. The presubiculum as well as deep layers of the medial EC of control animals showed an enhanced increase of fEPSP amplitudes within three to five minutes after applying the β-AR agonist. After isoproterenol application, control animals showed a stable LTP of evoked fEPSPs in the distal subiculum, presubiculum and deep layers of the medial EC for at least 50 min (presubiculum control: 33.810.3%, $p<0.01$, n=9, Fig. 16C; medial EC control: 58.311.7%, $p<0.01$, n=9, Fig. 16D).

In epileptic tissue the fEPSP facilitation was unaffected in the medial EC, while we observed a considerable loss of facilitated fEPSPs in the presubiculum (presubiculum pilocarpine: 3.42.4%, p=0.1, n=7, Fig. 16C; medial EC pilocarpine: 51.512.8%, $p<0.01$, n=7, Fig. 16D). I/O curves for parahippocampal target regions can be seen in Figure 16A and B.

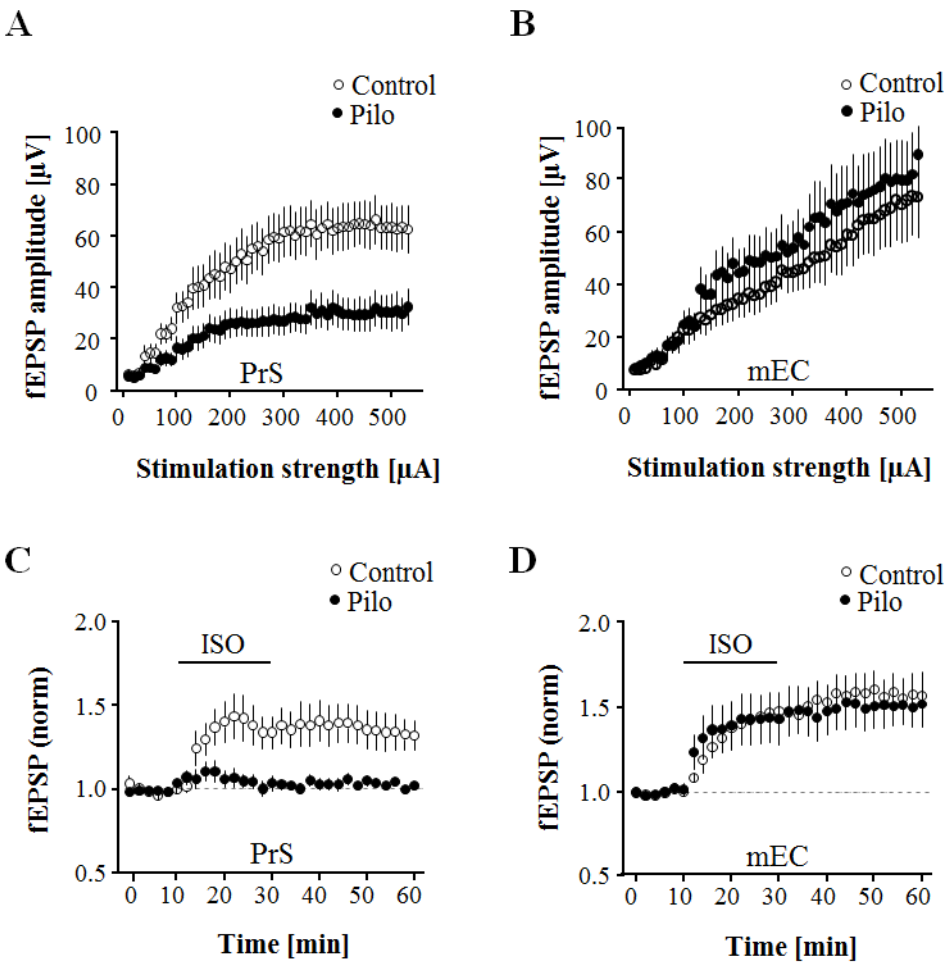

Figure 16: *(A,B) Input-output curves derived from the presubiculum and the medial entorhinal cortex for both animal groups.(C,D) Averaged time courses of normalized fEPSPs in the presubiculum and the medial entorhinal cortex of control and pilocarpine-treated animals after application of isoproterenol (10 M). Note the substantial decline of LTP in the presubiculum and the preserved LTP in the medial EC of pilocarpine-treated animals.*

5.2.2 Representation of Functional Connectivity between the Hippocampus and its Parahippocampal Targets in 3D

Upon investigating isoproterenol induced alterations of subicular fEPSPs and identifying target regions of facilitated synaptic transmission, we aimed to

depict changes of fEPSP amplitudes over time. A summary of the percental increase of fEPSP amplitudes under isoproterenol application in the subiculum and its target regions in control and pilocarpine treated animals is illustrated in Figure 17A. Based on these data we generated 3D plots by using a modified version of MEA-Tools [57]. The depicted examples in Figure 17 are individual sample slices, which represent the relative change of fEPSP amplitudes in control and pilocarpine-treated animals, respectively.

Plots were generated in false colors (control: dark red: approximately 162% increase of fEPSP amplitudes; pilocarpine: dark red: approximately 18% increase of fEPSP amplitudes; both: light blue: 0% increase of fEPSP amplitudes). The correlation between peaks of the 3D plots and regions of interest are shown in Figure 17B. Figures 17C a and d illustrate fEPSPs of the normalized baseline amplitudes. Within three to five minutes after application of isoproterenol, the fEPSP amplitudes of the distal subiculum increased and an enhancement of fEPSP amplitudes in distinct regions of the parahippocampus followed subsequently (Figure 17C, b and e). Peak changes of fEPSPs were observed in the deep layers of the mEC and the presubiculum. Smaller changes were located in the lateral entorhinal cortex (lEC) and superficial layers of the mEC. This region-specific facilitation of fEPSP amplitudes lasted for at least 50 minutes and was even slightly increasing in some regions after wash out of isoproterenol, as the distal subiculum and the mEC (Figure 17C, c and f).

In pilocarpine-treated animals there was only a slight increase of fEPSP amplitudes in the mEC, while in all other regions we observed no fEPSP facilitation (Figure 17C d-f).

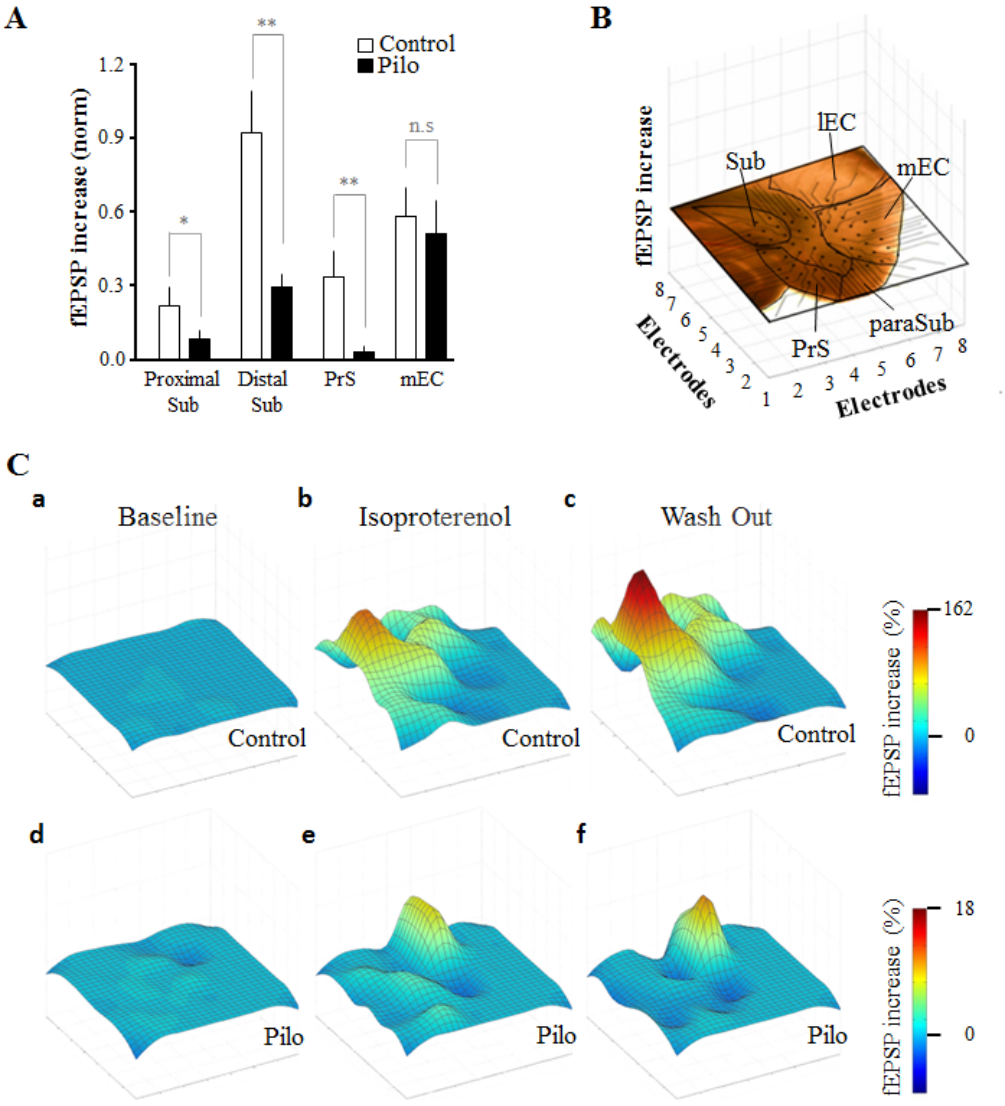

Figure 17: (A) Summary of subicular LTP induced by β-AR activation and its consequences for specific parahippocampal target structures. (B) Scheme to support the anatomical correlation between regions of interest and peaks in the color-coded 3D plots. (C) Illustration of 3D color-coded fEPSP detection. Note the circumscribed color-coded increase of fEPSPs in the distal subiculum (Sub), the presubiculum (PrS) and the medial entorhinal cortex (mEC) of control animals. X- and Y-axis display the MEA electrode grid while the z-axis displays the normalized increase in fEPSP amplitudes.

5.3 Efferent Stimulation reveals Facilitated Polysynaptic Transmission between CA1, the Subiculum and Parahippocampal Target Structures

Since a pronounced isoproterenol-induced LTP was only found in the distal part of the subiculum, we hypothesized , that the facilitated activation in parahippocampal target structures, such as the mEC and the presubiculum, might be a result of a facilitated polysynaptic transmission between CA1, the subiculum and its parahippocampal target structures. This hypothesis demanded a more detailed investigation, since the facilitated activation in parahippocampal target structures might also be a consequence of isoproterenol-induced LTP, generated intrinsically in the presubiculum and mEC.

5.3.1 β-AR Activation in Parahippocampal Target Structures at the Cellular Level

To test this hypothesis, we examined the effect of β-AR activation in parahippocampal target regions at the cellular level by placing the stimulation electrode in proximity to efferent pathways between subiculum and presubiculum and recording EPSCs from the presubiculum and layer V of the medial EC (Figure 18A). In contrast to subicular neurons, principal cells of the presubiculum and mEC were not effected by isoproterenol application. In control animals, neither membrane potential nor input resistance were modified by β-AR activation (presubiculum: I hold: 29.120.8%, p=n.s., n=6; input resistance: -4.33.0%, p=n.s., n=8; medial EC: I hold: -15.315.8%, n=5, p=n.s., input resistance: -5.97.6%, p=n.s., n=7). The same characteristics were found in presubicular cells and neurons of the medial EC in epileptic tissue (presubiculum: I hold: -5.910.3%, p=n.s., n=5, input resistance: 2.17.1%, p=n.s., n=5; medial EC: I hold: 6.76.0%, p=n.s., n=4, input resistance: -5.423.6%, p=n.s., n=4).

In addition, the application of the β-AR agonist isoproterenol had no ef-

fect on the modulation of EPSC amplitudes in the specific target regions, neither in control, nor in epileptic tissue (presubiculum control: EPSC increase: -2.68.9%, p=n.s., n=8, Figure 18C; medial EC control: EPSC increase: -5.310.0%, p=n.s.; n=7, Figure 18D; presubiculum pilocarpine: EPSC increase: -4.94.1%, p=n.s., n=5, Figure 18C; medial EC pilocarpine: EPSC amplitudes: 2.213.2%, p=n.s.; n=4, Figure 18D).

These results indicate that β-AR activation has no direct effect at the cellular level of presubicular and mEC principal neurons.

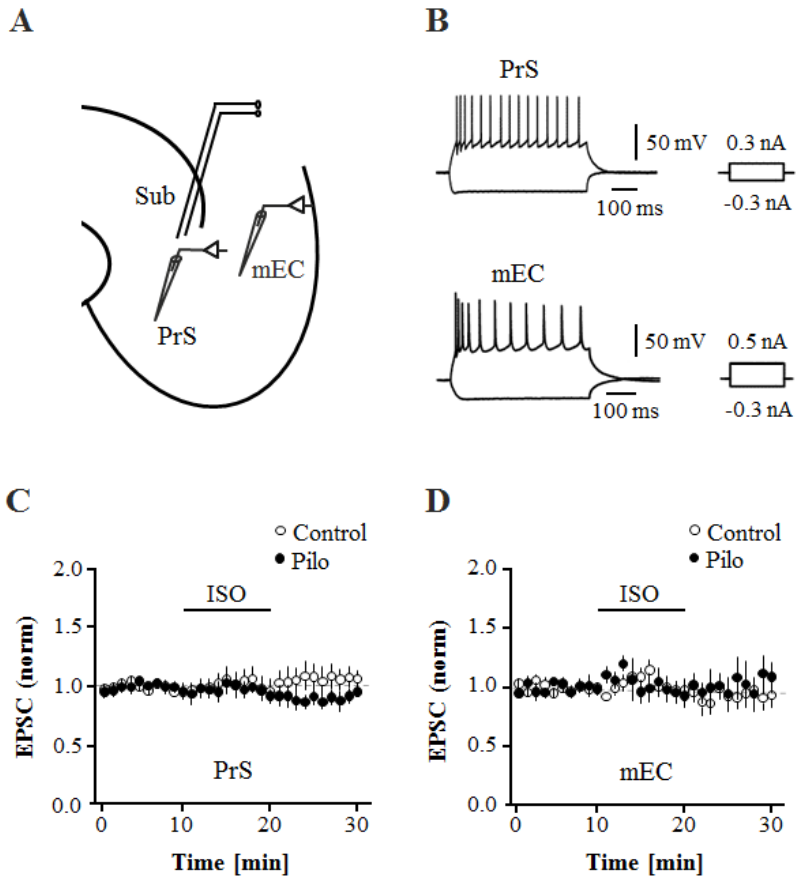

Figure 18: *(A) Scheme of stimulation and recording sites with the stimulation electrode being positioned between the distal subiculum and presubiculum (to stimulate efferent pathways) and recording electrodes in parahippocampal target structures. Hippocampal areas: Sub: subiculum, PrS: presubiculum, mEC: medial entorhinal cortex. (B) Representative voltage responses of principal neurons in both hippocampal target regions examined to depolarizing and hyperpolarizing current pulses. (C,D) Average time courses of evoked EPSC amplitudes under β-AR activation. Note, that isoproterenol failed to induce LTP in the presubiculum and the mEC.*

5.3.2 β-AR Activation in Parahippocampal Target Structures at the Network Level

Next we studied β-AR activation in parahippocampal target structures at the network level by applying alternating stimulation (stimulus interval: 15 s) to CA1-subiculum synapses (afferent stimulation) and the subicular efferents terminating in the presubiculum and deep layers of the medial EC (efferent stimulation) (see Figure 19A). By stimulating efferent pathways we are able to investigate, whether facilitated activation in parahippocampal target structures is intrinsically generated in the presubiculum and medial EC by β-AR activation.

As expected, application of the β-AR agonist isoproterenol in combination with the stimulation of CA1-subiculum synapses resulted in a stable LTP of evoked fEPSPs in both the presubiculum and the mEC (presubiculum afferent: 24.13.9%, $p<0.01$, n=7, Figure 19B; medial EC afferent: 31.16.8%, $p<0.01$, n=7, Figure 19C). In contrast, stimulation of subicular efferents failed to induce LTP in subicular target regions (presubiculum efferent: -6.74.9%, p=n.s., n=7, Figure 19B; medial EC efferent: 7.86.4%, p=n.s., n=7, Figure 19C).

In epileptic tissue, stimulation of the afferent pathway resulted only in an increase of fEPSP amplitudes in the medial EC, while presubicular fEPSPs remained unaffected (presubiculum: -3.513.0%, p=n.s., n=8, Figure 19D; medial EC: 24.34.6%, $p<0.05$, n=3, Figure 19E). Similar to control animals, efferent stimulation in pilocarpine-treated animals had no effect on fEPSP amplitudes in both target regions (presubiculum: 0.87.5%, p=n.s., n=8, Figure 19D; medial EC: -2.85.9%, p=n.s., n=3, Figure 19E).

Based on these results we can conclude that the facilitation of fEPSPs in the presubiculum and deep layers of the medial EC originates in isoproterenol-induced LTP at distal CA1-subiculum synapses.

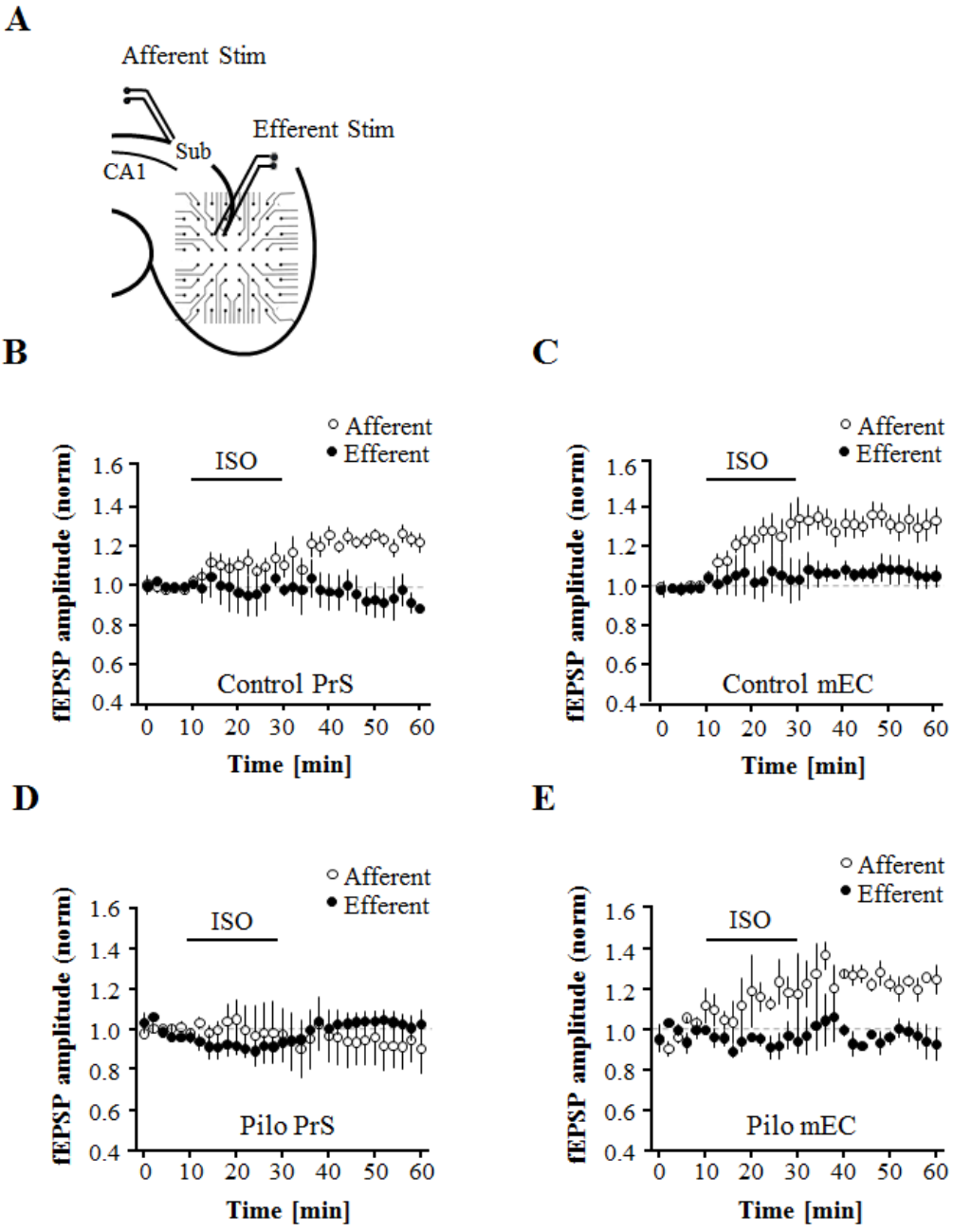

Figure 19: *(A) Scheme of a hippocampal brain slice on a MEA grid with the positioning of two stimulation electrodes. (B,C, D, E) Averaged time courses of normalized fEPSPs in subicular target regions of control and pilocarpine-treated animals.*

In summary, chemical LTP induced in the subiculum by β-AR activation is causing an increase of fEPSP amplitudes in subicular target regions via polysynaptic transmission between CA1, the subiculum and parahippocampal target structures. These results suggest that in control tissue isoproterenol-induced LTP at distal CA1-subiculum synapses causes a facilitation of fEPSPs in the presubiculum and deep layers of the medial EC. In epileptic tissue, this polysynaptic facilitation is preserved in the medial EC but attenuated in the presubiculum.

6 Discussion

The aim of the present study was to investigate how hippocampal output is modulated by β-adrenoceptor activation. Moreover differences in the modulation of hippocampal output by β-adrenoceptor activation were examined in the pilocarpine model of epilepsy. For this purpose we combined patch-clamp recordings, providing information about neuronal characteristics on a single cell level, with multi-electrode array recordings, picturing network plasticity and connectivity between hippocampal and parahippocampal structures.

We first confirmed previous results of our group by showing that β-AR activation by isoproterenol induces a chemical form of LTP in BS but not in RS cells of the subiculum [26]. The magnitude of LTP in BS neurons obtained from control animals was thereby correlated to the magnitude of LTP in BS neurons obtained from pilocarpine-treated animals. A facilitation of evoked EPSCs by β-AR activation however, could not be detected in subicular RS neurons in either animal groups. Hence the β-AR-dependent LTP in BS neurons is caused by a target-specific mechanism of CA1 efferents onto subicular pyramidal cells.

As shown by Wojtowicz et al., this -adrenergic-induced LTP does not require an increase in postsynaptic Ca^{2+} concentration. Subicular BS cells have been shown to require an increase of presynaptic calcium concentration for the expression of a presynaptic, activity-dependent LTP. The increase of presynaptic calcium in turn requires an increase of the secondary messenger cAMP and activates protein kinase A (PKA) [16, 63]. Isoproterenol application had no long-lasting effect on membrane properties such as resting membrane potential or input resistance in control and pilocarpine-treated animals. Both, the depolarization and the decrease of input resistance in BS and RS neurons during β-AR activation were transient and returned to baseline level after wash out of isoproterenol.

At the network level, β-AR-mediated subicular LTP was expressed in a region specific manner along the proximo-to-distal and deep-to-superficial axes of the subiculum and most prominent in the distal subiculum [7, 62, 8].

These results confirm the distinct spatial distribution of BS and RS neurons along the proximo-to-distal axis of the subiculum. Along this axis the density of BS neurons increases towards the distal region of the subiculum [52, 9, 8]. Hence, we found a potentiated β-AR-mediated LTP in the distal part of the subiculum, since LTP can only be induced in BS cells [26].

Though in pilocarpine-treated animals β-AR-mediated LTP is not impaired in BS neurons at the cellular level, we found a decreased β-AR-mediated LTP at the network level. Thus β-AR-mediated LTP is attenuated by pilocarpine treatment in the distal subiculum, where BS neurons represents the majority of excitatory pyramidal cells. This decrease of β-AR-mediated LTP might be explained by hippocampal sclerosis, the most common type of neuropathological damage seen in individuals suffering from TLE [64, 42]. Hippocampal sclerosis involves neuronal cell loss especially in the CA1 region and the subiculum of the hippocampus [42]. Thus the decreased number of BS cells could be a result of selective cell death. Another explanation could be an alteration of the intrinsic discharge properties of subicular BS cells by pilocarpine treatment. This might shift the relation of BS to RS neurons from 2:1 in controls to 1:2 in pilocarpine treated animals and might account for the decreased number of BS cells [42]. Interestingly, in human hippocampi of patients suffering from TLE, a similar low fraction of BS cells was observed [4].

To study the effect of β-AR activation on the connectivity between CA1, the subiculum and its parahippocampal target structures in pilocarpine-treated animals in comparison to control animals we employed a MEA device. The parahippocampal region is includes pre- and parasubiculum, the entorhinal and perirhinal cortices, as well as the postrhinal (nonprimate mammalians) or parahippocampal cortex (in primates including human) [65]. The presubiculum and the medial EC receive projections exclusively from its distal portion of the subiculum [58, 12, 6, 66], whereas RS cells target the lateral EC [67, 13].

We first identified target regions showing facilitated fEPSP amplitudes

during β-AR activation and determined the presubiculum and deep layers of the mEC as structures with a facilitated synaptic transmission after β-AR activation. These findings seem to be consistent with the topography of subicular-parahippocampal projections. The topography of the subiculum suggests that BS and RS neurons project to different target regions receptively [68, 10]. Projections from proximal subicular neurons terminate predominantly in the lateral EC whereas projections of distal subicular neurons primarily terminate in the medial EC [5, 69]. Several studies showed that pilocarpine- and kainite-induced status epilepticus causes preferential loss of glutamatergic neurons sparing GABAergic neurons in layer III of the medial EC [70, 71]. Considering that the temporoammonic projection from the mEC to the hippocampus is characterized by a strong feed-forward inhibition, degeneration of this projection might enhance the excitability in its parahippocampal target structures.

The β-AR-mediated strengthening of CA1-subicular BS cell synapses seems to play an important role in the interaction between the hippocampal area CA1, the subiculum and distinct parahippocampal target structures. In control animals, we found a facilitated connectivity between CA1, the subiculum and the presubiculum as well as the mEC. This polysynaptic transmission, mediated by the subiculum, seems to be impaired in pilocarpine treated animals in a target-specific manner. Our findings reveal a loss of LTP in the distal subiculum of epileptic animals which results in a lack of facilitation of fEPSP amplitudes in the presubiculum. The polysynaptic transmission between CA1, the subiculum and the medial EC on the other hand seems to be preserved and is still facilitated after β-AR activation. Though both, the presubiculum and the medial EC receive projections from the distal subiculum, only the projection to the presubiculum lacks the β-AR-induced facilitation. This surprising finding may suggest that in particular subicular BS cells projecting to the presubiculum are vulnerable against seizures, decreased in number and as a result do not contribute to the facilitation of subicular output. This assumption is supported by the fact that the strong

bi-directional connectivity between the subiculum and the presubiculum may favor enhanced excitability and cell death as observed in the presubiculum of pilocarpine-treated animals [72]. Considering the substantial decline of postsynaptic fEPSPs in the presubiculum, a decrease in glutamatergic transmission at subiculum-presubiculum synapses or a decrease in postsynaptic excitability may account for this effect as well.

Parahippocampal structures are of major importance for mediating hippocampal-cortical interaction. In this context, NE is associated with states of arousal, affect, attention, learning and memory [73]. Under healthy conditions, the β-AR modulation of subicular BS cells may contribute to the target-specific processing of the hippocampal output associated with the states of arousal, affect or attention. In the pilocarpine model of epilepsy, the selective impairment of the β-AR-dependent modulation of information transfer from the hippocampus to its target structures (presubiculum) might contribute to hippocampus-dependent impairments, which are often recognized in patients suffering from TLE.

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7 Appendix

7.1 List of Publications

- E. Franzoni, S. A. Booker, S. Parthasarathy, F. Rehfeld, **S. Grosser**, S. Srivatsa, H. R. Fuchs, V. Tarabykin, I. Vida, F. G. Wulczyn, "miR-128 regulates neuronal migration, outgrowth and intrinsic excitability via the intellectual disability gene Phf6." *eLife* 10.7554/eLife.04263, January 2015. (2014 Impact factor: 8.52)

- **S. Grosser**, J.-O. Hollnagel, K. E. Gilling, J. C. Bartsch, U. Heinemann, J. Behr, "Gating of hippocampal output by -adrenergic receptor activation in the pilocarpine model of epilepsy." *Neuroscience* 286:325-337, February 2015. (2013 Impact Factor: 3.33)

- **S. Grosser**, B. N. Queenan, R. R. Lalchandani, S. Vicini, "Hilar somatostatin interneurons contribute to synchronized GABA activity in an in vitro epilepsy model." *Plos one* 9(1):e86250, January 2014. (2015 Impact factor: 3.53)

7.2 Symposia and Meeting Contribution

2011 NETWORKS! , Tbingen

Poster presentation: “Gating of hippocampal output by beta-adrenergic receptor activation”

Graduate student meeting of several neuroscientific graduate schools funded by the DFG

2012 BNF, Berlin

Poster presentation: “Gating of hippocampal output by beta-adrenergic receptor activation”

Berlin Neuroscience Forum

2012 MEA Meeting, Reutlingen

Meeting for users of multi-electrode Arrays and prospects

Poster presentation: “Gating of hippocampal output by beta-adrenergic receptor activation”

2012 FENS, Barcelona

Poster presentation. “Gating of hippocampal output by beta-adrenergic receptor activation”

2012 NETWORKS!, Berlin

Poster presentation and head of the organizing team: “Gating of hippocampal output by beta-adrenergic receptor activation”

Graduate student meeting of several neuroscientific graduate schools funded by the DFG

2013 5th G-Node Winter Course: Neural Data Analysis, Munich

4 day course organized by the Bernstein Network of Computational Neuroscience

2013 SfN, San Diego

Poster presentation: "Gating of hippocampal output by beta-adrenergic receptor activation in the pilocarpine model of epilepsy"